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**BIOLOGICAL INTEGRITY OF HUGHES CREEK
IN THE
BITTERROOT RIVER TMDL PLANNING AREA
BASED ON THE STRUCTURE AND COMPOSITION OF
THE BENTHIC ALGAE COMMUNITY**

Prepared for:

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Summary

On the Fourth of July, 2003, periphyton samples were collected from 2 sites on Hughes Creek in the Bitterroot River TMDL planning area in southwestern Montana for the purpose of assessing whether this stream is water-quality limited and in need of TMDLs. The samples were collected following MDEQ standard operating procedures, processed and analyzed using standard methods for periphyton, and evaluated following modified USEPA rapid bioassessment protocols for wadeable streams.

Diatom metrics at both sites on Hughes Creek indicated excellent biological integrity, no impairment, and full support of aquatic life uses. However, values for the pollution index at both sites approached the threshold for minor impairment and indicate elevated organic loading. This organic loading may be natural in origin.

A large amount of fine sand was noted in the sample from the lower site on Hughes Creek. However, values for the sedimentation index were well below the threshold for minor impairment in a mountain stream. Nevertheless, both sites did support large numbers of *Planothidium lanceolatum*, a diatom that is adapted to living on grains of sand.

The diatom and non-diatom algal floras of Hughes Creek indicate moderate gradients and current velocities with little disturbance, and cold, alkaline, and highly oxygenated waters with somewhat elevated levels of organic and inorganic nutrients. Nitrogen is probably the limiting nutrient in this stream. The two sites supported very similar floras, which indicates very similar ecological conditions. Diatom species richness, diversity, and equitability were excellent. No abnormal diatom cells were observed, which indicates the probable absence of toxic chemicals.

Introduction

This report evaluates the biological integrity¹, support of aquatic life uses, and probable causes of stress or impairment to aquatic communities in Hughes Creek in the Bitterroot River TMDL planning area in southwestern Montana. The purpose of this report is to provide information that will help the State of Montana determine whether Hughes Creek is water-quality limited and in need of TMDLs.

The federal Clean Water Act directs states to develop water pollution control plans (Total Maximum Daily Loads or TMDLs) that set limits on pollution loading to water-quality limited waters. Water-quality limited waters are lakes and stream segments that do not meet water-quality standards, that is, that do not fully support their beneficial uses. The Clean Water Act and USEPA regulations require each state to (1) identify waters that are water-quality limited, (2) prioritize and target waters for TMDLs, and (3) develop TMDL plans to attain and maintain water-quality standards for all water-quality limited waters.

Evaluation of aquatic life use support in this report is based on the species composition and structure of periphyton (benthic algae, phytobenthos) communities at two sites on Hughes Creek that were sampled on July 4, 2003. Periphyton is a diverse assortment of simple photosynthetic organisms called algae that live attached to or in close proximity of the stream bottom. Some algae form long filaments or large colonies and are conspicuous to the unaided eye. But most algae, including the ubiquitous diatoms, can be seen and identified only with the aid of a microscope. The periphyton community is a basic biological component of all aquatic ecosystems. Periphyton accounts for much of the primary production and biological diversity in Montana streams (Bahls et al. 1992). Plafkin et al. (1989) and Barbour et al. (1999) list several advantages of using periphyton in biological assessments.

¹ *Biological integrity* is defined as "the ability of an aquatic ecosystem to support and maintain a balanced, integrated, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of natural habitats within a region" (Karr and Dudley 1981).

Project Area and Sampling Sites

The project area is located within the Montana extension of the Idaho Batholith Ecoregion in Ravalli County, Montana. This ecoregion is mountainous, deeply dissected, partially glaciated, and characteristically underlain by granitic rocks. Soils derived from granitics are droughty and have limited fertility, and therefore provide only limited amounts of nutrients to aquatic systems (McGrath et al. 2001). Vegetation in the project area is mixed conifer forest at higher elevations and ponderosa pine, shrubs and grasses at lower elevations (USDA 1976, Woods et al. 1999). The main land uses are logging, grazing, recreation, mining, and wildlife production. Streams in this ecoregion are likely to suffer from increased loads of fine sediments after disturbance by humans. In the Idaho portion of this ecoregion, logging has caused slope instability (especially in granitic areas) and stream sedimentation. Placer gold mining has heavily affected rivers in this ecoregion in the state of Idaho (McGrath et al. 2001).

Periphyton samples were collected at two sites on Hughes Creek (Table 1). Elevation at the sampling sites is about 5,700 feet. Hughes Creek is an east-side tributary of the West Fork of the Bitterroot River upstream from Painted Rocks Reservoir. Hughes Creek and the West Fork of the Bitterroot River are headwater tributaries of the Bitterroot River in USGS hydrologic unit 17010205. The Bitterroot River is a tributary of the Clark Fork River. Hughes Creek is classified B-1 in the Montana Surface Water Quality Standards.

Methods

Periphyton samples were collected following standard operating procedures of the MDEQ Planning, Prevention, and Assistance Division. Using appropriate tools, microalgae were scraped, brushed, or sucked from natural substrates in proportion to the importance of those substrates at each study site. Macroalgae were picked by hand in proportion to their abundance at the site. All collections of microalgae and macroalgae were pooled into a common container and preserved with Lugol's (KI) solution.

The samples were examined to estimate the relative abundance and rank by biovolume of diatoms and genera of soft (non-diatom) algae according to the method described in Bahls (1993). Soft algae were identified using Smith (1950), Prescott (1962, 1978), John et al. (2002), and Wehr and Sheath (2003). These books also served as references on the ecology of the soft algae, along with Palmer (1969, 1977).

After the identification of soft algae, the raw periphyton samples were cleaned of organic matter using sulfuric acid, potassium dichromate, and hydrogen peroxide. Then permanent diatom slides were prepared using Naphrax, a high refractive index mounting medium, following *Standard Methods for the Examination of Water and Wastewater* (APHA 1998). At least 300 diatom cells (600 valves) were counted at random and identified to species. The following were the main taxonomic references for the diatoms: Krammer and Lange-Bertalot 1986, 1988, 1991a, 1991b; Lange-Bertalot 1993, 2001; Krammer 1997a, 1997b, 2002; Reichardt 1997, 1999. Diatom naming conventions followed those adopted by the Academy of Natural Sciences for USGS NAWQA samples (Morales and Potapova 2000) as updated in 2003 (Dr. Eduardo Morales, Academy of Natural Sciences, digital communication). Van Dam et al. (1994) was the main ecological reference for the diatoms.

The diatom proportional counts were used to generate an array of diatom association metrics. A metric is a characteristic of the biota that changes in some predictable way with increased human influence (Barbour et al. 1999). Diatoms are particularly useful in generating metrics because there is a wealth of information available in the literature regarding the pollution tolerances and water quality preferences of common diatom species (e.g., Lowe 1974, Beaver 1981, Lange-Bertalot 1996, Van Dam et al. 1994).

Values for selected metrics were compared to biocriteria (numeric thresholds) developed for streams in the Rocky Mountain ecoregions of Montana (Table 2). These criteria are based on metric values measured in least-impaired reference streams (Bahls et al. 1992) and metric values measured in streams that are known to be impaired by various sources and causes of pollution (Bahls 1993). The biocriteria in Table 2 are valid only for samples collected during the summer field season (June 21-September 21).

The criteria in Table 2 distinguish among four levels of stress or impairment and three levels of aquatic life use support: (1) no impairment or only minor impairment (full support), (2) moderate impairment (partial support), and (3) severe impairment (nonsupport). These impairment levels correspond to excellent, good, fair, and poor biological integrity, respectively. In cold, high-gradient mountain streams, natural stressors will often mimic the effects of man-caused impairment on some metric values.

Quality Assurance

Several steps were taken to assure that the study results are accurate and reproducible. Upon receipt of the samples, station and sample attribute data were recorded in the Montana Diatom Database and the samples were assigned a unique number, e.g., 2953-01. The first part of this number (2953) designates the sampling site (Hughes Creek above Thunder Mountain Road) and the second part (01) designates the number of periphyton samples that have been collected at this site for which data have been entered into the Montana Diatom Database.

Sample observations and analyses of soft (non-diatom) algae were recorded in a lab notebook along with information on the sample label. A portion of the raw sample was used to make duplicate diatom slides. The slide used for the diatom proportional count will be deposited in the Montana Diatom Collection at the University of Montana Herbarium in Missoula. The duplicate slide will be retained by *Hannaea* in Helena. Diatom proportional counts have been entered into the Montana Diatom Database.

Results and Discussion

Results are presented in Tables 3, 4 and 5, which are located near the end of this report following the references section. Appendix A contains a series of diatom reports, one for each sample. Each diatom report contains an alphabetical list of diatom species in that sample and their percent abundances, and values for 65 different diatom metrics and ecological attributes.

Sample Notes

Hughes Creek below first FS gate. Clean sample, little sediment is present.

Hughes Creek above Thunder Mountain Road. Sediment (~10 micron diameter fine sand) is extremely heavy.

Non-Diatom Algae (Table 3)

The two sites on Hughes Creek supported very similar algal floras consisting of green algae, cyanobacteria, diatoms, and a chrysophyte. The lower site supported three more genera of non-diatom algae than the upper site. A downstream increase in the number of non-diatom algal genera and diatom species is the normal pattern in mountain streams.

The sample collected at the upper site on Hughes Creek (below first gate on FS parcel) was dominated by a filamentous green alga (*Ulothrix*) and a branched cyanobacterium with heterocysts (*Tolypothrix*)(Table 3). These cold-water algae are common in mountain streams. *Tolypothrix* is capable of fixing atmospheric (molecular) nitrogen, which is an advantage in waters that are poor in nitrogen. Diatoms were abundant at this site and ranked 3rd in biovolume. The chrysophyte *Hydrurus foetidus* ranked 4th and was rare.

The sample collected at the lower site on Hughes Creek was also dominated by *Ulothrix*. The cyanobacterium *Nostoc* ranked 2nd in biovolume here and was a co-dominant with *Ulothrix*. Like *Tolypothrix*, *Nostoc* has heterocysts, is a nitrogen fixer, and prefers cold waters. Diatoms were abundant and ranked 3rd in biovolume at the lower site. Cells of the chrysophyte *Hydrurus foetidus*, which forms slimy mucilaginous masses, were frequent and this genus ranked 4th in biovolume at this site. Wehr and Sheath (2003) describe *Hydrurus foetidus* as follows:

One of the most dramatic examples of a cold-water stenotherm is the mountain-stream dwelling chrysophyte *Hydrurus foetidus*. This macroscopic, brown, gelatinous, unpleasant-smelling alga is relatively abundant in both the eastern and western mountain

streams of North America. The gelatinous envelope in which the cells are embedded is exceedingly tough and the plant frequently covers the entire surface of submerged rocks and has caused more than one hiker to lose his or her footing when crossing a stream. It normally begins to disappear when water temperatures rise much above 10°C...Other requirements for this species apparently include low pH and bright sunlight.

The nitrogen-fixing cyanophyte *Tolyphothrix* was frequent at the lower site and ranked 5th, followed by the branched filamentous green alga *Stigeoclonium*, which was also frequent. *Stigeoclonium* is tolerant of organic pollution and its presence may indicate an increase in organic loading at this site.

Diatoms (Table 4)

All of the major diatom species from Hughes Creek are included in pollution tolerance classes 3 or 2, and are either sensitive to organic pollution or only somewhat tolerant of organic pollution (Table 4).

Diatom metrics for both sites on Hughes Creek indicate excellent biological integrity, no impairment, and full support of aquatic life uses (Table 4). However, pollution index values at both sites (2.53 and 2.54) approached the threshold for minor impairment in a mountain stream (2.50). This slight organic loading may be natural in origin and due to an accumulation of terrestrial plant debris and/or algae. Several of the major diatom species (e.g., *Diatoma mesodon*, *Fragilaria spp.*, *Hannaea arcus*, *Staurosira construens*, *Synedra spp.*) are free-living and indicate a history of stable flows at these sites and the absence of recent bottom-scouring events. These diatoms also indicate cold waters.

Although sedimentation index values did not exceed the threshold for minor impairment at either site, a large amount of fine sand was noted in the sample collected at the lower site (see sample notes, above). This site also had the larger sedimentation index value of the two sites based on the percentage of motile diatoms that were counted. In addition to motile diatoms, these sites supported large numbers of *Planothidium lanceolatum*, a diatom species that is adapted to living attached to grains of sand. If the percentage of *Planothidium lanceolatum* at

these sites is added to the percentage of motile diatoms, sedimentation index values would approach but still not exceed the threshold for minor impairment (Table 4).

Both sites on Hughes Creek supported diatom assemblages with excellent species richness, diversity, and equitability for a mountain stream. The relatively small percentage of *Achnanthidium minutissimum* at both sites indicates a stream with moderate gradient and current velocity and little physical disturbance. The absence of teratological (abnormal) cells indicates that toxicity from heavy metals is not likely to be a problem here.

As with the non-diatom algae, the two sites on Hughes Creek supported very similar diatom assemblages. The percent community similarity between the two sites was 60.35, which indicates very similar floras and ecological conditions. Adjacent sites on the same stream without intervening tributaries or point source discharges typically share 60 percent or more of their diatom assemblages (Bahls 1993).

Modal Categories of Ecological Attributes (Table 5)

Several ecological attributes assigned by Stevenson and Van Dam et al. (1994) were selected from the diatom reports in the appendix and modal categories of these attributes were extracted to characterize water quality tendencies in Hughes Creek (Table 5). Most of the diatoms that inhabit Hughes Creek may be characterized as non-motile, alkaliphilous, and autotrophic, while tolerating high levels of organic nitrogen and a moderate amount of organic loading. They prefer meso-eutrophic to eutrophic, fresh waters, and exert a continuously high demand for dissolved oxygen. These categories are defined by Van Dam et al. (1994).

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Table 1. Location of MDEQ periphyton sampling stations on Hughes Creek in 2003.

Station	Montana DEQ Station Code	Hannaea Sample Number	Latitude	Longitude	Sample Date
Hughes Creek below 1st gate on FS	C05HUGH01	2952-01	45 35 58	114 09 40	7/4/03
Hughes Creek above Thunder Mtn. Road	C05HUGH02	2953-01	45 36 28	114 17 08	7/4/03

Table 2. Diatom association metrics used by the State of Montana to evaluate biological integrity in mountain streams: references, range of values, expected response to increasing impairment or natural stress, and criteria for rating levels of biological integrity. The lowest rating for any one metric is the rating for that site.

Biological Integrity/ Impairment or Stress/ Use Support	No. of Species Counted ¹	Diversity Index ² (Shannon)	Pollution Index ³	Siltation Index ⁴	Disturbance Index ⁵	% Dominant Species ⁶	% Abnormal Cells ⁷
Excellent/None Full Support	>29	>2.99	>2.50	<20.0	<25.0	<25.0	0
Good/Minor Full Support	20-29	2.00-2.99	2.01-2.50	20.0-39.9	25.0-49.9	25.0-49.9	>0.0, <3.0
Fair/Moderate Partial Support	19-10	1.00-1.99	1.50-2.00	40.0-59.9	50.0-74.9	50.0-74.9	3.0-9.9
Poor/Severe Nonsupport	<10	<1.00	<1.50	>59.9	>74.9	>74.9	>9.9
References	Bahls 1979 Bahls 1993	Bahls 1979 Bahls 1993	Bahls 1993 Bahls 1993	Bahls 1993 Bahls 1993	Barbour et al. 1999 Barbour et al. 1999	Barbour et al. 1999 Barbour et al. 1999	McFarland et al. 1997
Range of Values	0-100+	0.00-5.00+	1.00-3.00	0.0-90.0+	0.0-100.0	~5.0-100.0	0.0-30.0+
Expected Response	Decrease ⁸	Decrease ⁸	Decrease	Decrease	Increase	Increase	Increase

¹Based on a proportional count of 400 cells (800 valves)

²Base 2 [bits] (Weber 1973)

³Composite numeric expression of the pollution tolerances assigned by Lange-Bertalot (1979) to the common diatom species

⁴Sum of the percent abundances of all species in the genera *Navicula*, *Nitzschia* and *Surirella*

⁵Percent abundance of *Achnanthidium minutissimum* (synonym: *Achnanthes minutissima*)

⁶Percent abundance of the species with the largest number of cells in the proportional count

⁷Cells with an irregular outline or with abnormal ornamentation, or both

⁸Species richness and diversity may increase somewhat in mountain streams in response to slight to moderate increases in nutrients or sediment

Table 3. Relative abundance of cells and ordinal rank by biovolume of diatoms (Division Bacillariophyta) and genera of non-diatom algae in periphyton samples collected from Hughes Creek in 2003.

Taxa	Hughes Creek 01 C05HUGH01	Hughes Creek 02 C05HUGH02
Cyanophyta (cyanobacteria)		
<i>Nostoc</i>		dominant/2nd
<i>Tolyphothrix</i>		frequent/5th
Chlorophyta (green algae)		
<i>Cladophora</i>		common/7th
<i>Chlorococcum</i>		frequent/6th
<i>Ulothrix</i>		dominant/1st
Chrysophyta (yellow-green algae)		
<i>Hydrurus foetidus</i>	rare/4th	frequent/4th
Bacillariophyta (diatoms)		
# Non-Diatom Genera	3	abundant/3rd
		6

Table 4. Percent abundance of major diatom species¹ and values of selected diatom association metrics for periphyton samples collected from Hughes Creek in 2003. Underlined values indicate minor stress; bold values indicate moderate stress; underlined and bold values indicate severe stress; all other values indicate no stress and full support of aquatic life uses when compared to criteria for mountain streams in Table 2. Stress may be natural or anthropogenic (see text).

Species/Metric	PTC ²	Hughes Creek 01 C05HUGH01	Hughes Creek 02 C05HUGH02
<i>Achnanthidium minutissimum</i>	3	4.55	4.68
<i>Diatoma mesodon</i>	3	10.66	1.25
<i>Encyonema silesiacum</i>	2	4.70	0.31
<i>Fragilaria capucina</i>	2	0.31	4.84
<i>Fragilaria vaucheriae</i>	2	10.34	10.14
<i>Gomphoneis eriense</i>	3	0.63	4.37
<i>Gomphonema olivaceooides</i>	3	8.15	5.30
<i>Hannaea arcus</i>	3	5.17	2.50
<i>Nitzschia dissipata</i>	3	3.61	6.71
<i>Planothidium lanceolatum</i>	2	8.46	3.43
<i>Staurosira construens</i>	3	12.07	17.94
<i>Synedra acus</i>	2	0.78	3.59
<i>Synedra ulna</i>	2	7.05	11.54
Number of Species Counted	41	48	
Shannon Species Diversity	4.36	4.39	
Pollution Index	2.54	2.53	
Siltation Index	9.56	14.20	
Disturbance Index	4.55	4.68	
Percent Dominant Species	12.07	17.94	
Percent Abnormal Cells	0.00	0.00	
Similarity Index ³		60.35	

¹A major diatom species accounts for 3.0% or more of the cells at one or more stations in a sample set.

²(Organic) Pollution Tolerance Class (Lange-Bertalot 1979): 1 = most tolerant; 2 = tolerant; 3 = sensitive.

³Percent Community Similarity (Whittaker 1952) when compared to the diatom assemblage at the adjacent upstream station

Table 5. Modal categories for selected ecological attributes of diatom species in Hughes Creek.

Ecological Attribute	Hughes Creek 01 C05HUGH01	Hughes Creek 02 C05HUGH02
Motility ¹	Not Motile	Not Motile
pH ²	Alkaliphilous	Alkaliphilous
Salinity ²	Fresh	Fresh
Nitrogen Uptake ²	Autotrophs (tolerate high organics)	Autotrophs (tolerate high organics)
Oxygen Demand ²	Continuously High	Continuously High
Saprobity ²	beta-Mesosaprobous	beta-Mesosaprobous
Trophic State ²	Eutraphentic	Meso-eutraphentic

¹Dr. R. Jan Stevenson, Michigan State University, digital communication.

²Van Dam et al. 1994